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Predominance and Antibiotic Susceptibility Profiles of Non-Typhoidal Bacteria Species in Blood Samples of Individuals Diagnosed of Typhoid Fever in Abakaliki

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Abstract **Original Research Article**

Blood stream infections are commonly associated with prolonged hospitalization of critically ill patients. Most of the cases are infamously associated with members of Enterobacteriaceae family which is also responsible for typhoid fever endemic in Nigeria. In this study, blood samples collected through vein puncture, were obtained from 120 subjects at three (3) different laboratories within Abakaliki metropolis, Ebonyi state. They were screened for typhoid using a widal test kit. Samples with significant titre values were inoculated into Brain heart infusion broth. The broth cultures were incubated for 48 hours at 37°C with intermittent mixing; subcultured onto xylose lysine deoxycholate agar, and incubated under the same condition. The isolates were identified using serological and biochemical tests. Antimicrobial susceptibility tests of the isolates involved the use of Kirby-Bauer method. The results showed that 49 out of 120 patients recorded a significant titre value. Out of 49 patients, bacteremia associated with Enterobacteriaceae was reported in five male patients (20.8%) and nine female patients (36%). Among the bacterial isolates belonging to Enterobacteriaceae, *Proteus vulgaris* had the highest prevalence (42.9%), followed by *S. typhi* (25.4%), then *S. enteritidis* (14.3%), while the least were *S. typhimurium, S. paratyphi* A, and *S. paratyphi* B (7.1% each). *Salmonella* species were sensitive to all the antibiotics tested, whereas *P. vulgaris* showed resistance to nalidixic acid, ceporex, and ampicillin. For proper diagnosis of typhoid fever, standard culture methods and antibiotic susceptibility test are recommended as a routine laboratory practice in addition to widal test. The result will guide medical treatment and reduce the spread of antibiotic resistance.

Keywords: Antibiotic resistance, bacteremia, blood stream infections, opportunistic pathogen, widal test.

INTRODUCTION

Globally, it is estimated that between 128, 000 - 161, 000 persons lose their lives out of 11 to 20 million cases of typhoid fever reported annually (Mujahid *et al*., 2020). The etiologic agent responsible for typhoid is *Salmonella enterica*. A total of 6 subspecies are grouped under *S. enterica*, while the serovars are close to 3, 000. The serovars are conveniently divided into non-typhoid and typhoid groups. The latter include *Salmonella typhi, Salmonella paratyphi,* and *Salmonella sendai* (Eng *et al*., 2015; Worley, 2023). *Salmonella enterica* serotype Typhi is responsible for typhoid, while *Salmonella enterica* serotypes Paratyphi A, B, or C are responsible for paratyphoid fever (Keerthana *et al*., 2018; Dutta *et al*., 2020). Headache, diarrhoea (or constipation), and abdominal pain are usually experienced by patients before fever manifest (Eng *et al*., 2015). Lack of specific symptoms of typhoid fever makes it necessary to carry out screening test of patients that complained they have experienced fever that lasted for one week, especially in endemic areas (Sattar *et al.,* 2014). According to Mawazo *et al.,* (2019), bone marrow, blood, and stool culture are employed in the diagnosis of typhoid fever. The use of culture media to isolate the bacterium responsible for typhoid fever takes between 2-7 days. Due to high cost and long period of laboratory analysis, rapid diagnostic methods which include Widal, TUBEX, TPTest, and Typhidot have been developed (Gaikwad and Rajurkar, 2014; Muhie *et al*., 2023).

Healthy individuals are at risk of being infected by carriers of *Salmonella* Typhi and patients clinically diagnosed with typhoid fever. It happens when food and water contaminated with stools, urine, and vomitus of persons infected by *Salmonella typhi* and *S. paratyphi* are ingested by non-carriers of the pathogen (Muhie *et al*., 2023). The bacterium in large numbers affect human

organs after spreading from the intestine to the bloodstream (Osue *et al*., 2022). Co-infection of typhoid and paratyphoid was reported by Dutta *et al.,* (2020). *Salmonella* infection is most commonly manifested as gastroenteritis, less frequently as bacteremia, and then enteric fever (Eng *et al*., 2015). *Salmonella* spp. is frequently isolated from blood culture of children between 0-18 years, living in sub-Saharan Africa (Dutta *et al.,* 2020). According to Eng *et al.,*(2015), paratyphoid fever and typhoid fever are collectively known as 'enteric fever' because their symptoms are difficult to distinguish, while the pathogens responsible for both disease conditions is called typhoid *Salmonella*. This bacteria belong to the Enterobacteriaceae family (Oludairo *et al*., 2022).

Enterobacteriaceae is a remarkable group of prokaryotic bacteria of public health concern. Gram-negative bacilli which are members of Enterobacteriaceae family that possess various characteristics have the largest population, compared with other groupings (dos Santos *et al.,* 2015; Janda and Abbott, 2021). In the past few decades, Enterobacteriaceae family has undergone several taxonomic changes. According to Morales-López *et al.,*(2019), the Enterobacteriaceae family comprise of 32 genera. They are natural inhabitants of the intestinal tract of humans and animals. Other environments where they can be found include soil, water, and plants (Morales-López *et al*., 2019; Al-Omari *et al*., 2022). Members of Enterobacteriaceae are implicated in bacterial blood stream infections (BSI) which poses a serious threat to public health in mainly developing countries, where the number of deaths associated with the infection is a source of concern (Duru *et al.,* 2020).

Resistance of typhoidal *Salmonella* and other members of Enterobacteriaceae family to commonly used antibiotics for treatment is a serious threat to public health (Faisal *et al*., 2021). There are reports on extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (Bindayna and Murtadha, 2011; Sangare *et al*., 2016), and emerging carbapenem-resistant *Enterobacteriaceae* infection (Tilahun *et al*., 2021). In north central Nigeria, Uzairue *et al.,*(2023) reported antimicrobial resistance and virulence genes in *Salmonella enterica* isolated from children diagnosed with bacteremia in northern Nigeria. In Semarang, Enterobacteriaceae family which include *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Enterobacter cloacae*, and *Salmonella typhi* were isolated from widal blood cultures (Darmawati *et al*., 2014). Although several studies on typhoid fever in patients have been reported, there is limited information on clinically diagnosed patients with Enterobacteriaceae infections. Therefore, this study is aimed at detecting opportunistic Enterobacteriaceae in the blood of typhoidal patients infected with *Salmonella typhi* and *S. paratyphi*.

MATERIALS AND METHODS

Study area

The study was carried out in three (3) Medical Laboratories in Abakiliki metropolis, Ebonyi state.

Ethical consideration

Ethical considerations for investigating systemic Enterobacteriaceae infections in typhoid fever encompass acquiring informed permission, safeguarding participant anonymity, and mitigating risks while optimizing benefits. Participation must be freely done, equitable, and consistent with scientific and social value. The study must undergo evaluation and approval by an ethics review board to protect the rights and welfare of participants.

Sample collection

A total of 120 venous blood samples were randomly collected from patients suspected to be suffering from typhoid fever. The subjects were 60 males and 60 females within the ages of 0-40 years. Three milliliters (3 ml) of blood was aseptically drawn from each of the patients. The samples were transferred into EDTA labeled containers and centrifuged to obtain the serum.

Widal test

Widal test (quantitative test) was performed on each serum samples obtained from 120 subjects using the procedure described by Jemilohun *et al.,* (2017).

Isolation and characterization of Enterobacteriaceae

The samples that recorded a significant widal test were inoculated into brain heart infusion broth (Sifin Diagnostics, Germany). A minimum of blood to broth ratio of 1 to 4 was maintained. The inoculated blood culture broth was incubated at 37 ^{o}C with intermittent mixing for 48 hours. The broth blood cultures were subcultured onto xylose lysine deoxycholate (XLD) agar: Titan Biotechnology, India) and incubated for 48 hours at 37 ^{o}C. The isolates were subjected to biochemical tests which include triple sugar iron agar (TSI), sugar fermentation, oxidase, motility, methyl red-Voges Proskauer, catalase, indole, starch hydrolysis, urease, and citrate test (Abdullahi, 2010). Serological tests as described by Abdullahi (2020) was performed to identify the *Salmonella* spp.

Antibiotic susceptibility test

The Kirby Bauer disc diffusion method described by Abdullahi, (2010) was used to perform antibiotic susceptibility test of the identified bacteria isolates belonging to Enterobacteriaceae family. The diameter of zone of clearance/inhibition was measured using a meter ruler and the result was recorded.

RESULTS

Table 1 shows the distribution of significant widal titre value and serum samples that also showed microbial growth in blood culture. The result shows that 49 out of 120 serum samples obtained from the subjects had a significant widal titre value. Among the 49 serum samples tested, 14 showed a microbial growth in broth blood culture, whereas 35 serum samples did not.

Table 1: Distribution of significant widal titre values and bacterial growth in blood culture (n=120)

Bacterial species	No. of significant widal titre value	Significant widal titre + bacterial growth in blood culture
Typhoidal species		5/14 (35.7%)
Non-typhoidal species		9/14 (64.3%)
Total	49 (40.8%)	14 (28.6%)

Presented in Table 2 is the distribution of Enterobacteriaceae isolated from the serum of patients that recorded a significant widal titre value. The result shows that the number of females (9) that had a significant widal titre value was higher than the males (5). *Proteus vulgaris* and *Salmonella typhi* was found in the serum of both male and female patients.

Table 2: Distribution of Enterobacteriaceae isolated from blood that recorded a significant widal titre value and bacterial growth in blood culture

Gender	No. of significant widal value	No. of significant widal titre value and bacterial growth in blood culture	Bacterial species (No. of isolates)
Male	24	5 (20.8%)	*Salmonella paratyhpi* B (1) *Salmonella typhimurium* (1) *Proteus vulgaris* (1) *Salmonella. typhi* (2)
Female	25	9 (36%)	*Salmonella. paratyphi* A (1) *Proteus vulgaris* (5) *Salmonella. enteritidis* (2) *Salmonella. typhi* (1)
Total	49	14 (28.6%)	

Table 3 shows the age distribution of the patients whose serum recorded a significant widal titre value and microbial growth in the blood culture. The result shows that the patients between 21-30 years old had the highest number (40) of significant widal titre value. Also within the same age bracket, the highest number of serum samples from 14 patients recorded a microbial growth in blood culture in addition to significant widal titre value.

Depicted in Figure 1 is the prevalence of bacteria belonging to Enterobacteriaceae family isolated from serum of typhoid patients. The result shows that *Proteus vulgaris* had the highest prevalence (42.90%), while the least involved *Salmonella typhimurium*, *Salmonella typhi* A, and *S. typhi* B (7.10% each).

Table 4 shows the antibiotic susceptibility profile of bacteria belonging to Enterobacteriaceae family isolated from serum of individuals that recorded a significant widal titre value. The result shows that all the isolates were sensitive to the antibiotics tested with the exception of *Proteus vulgaris* which was resistant to ceporex, nalidixic acid, and ampicillin.

Table 3: Age distribution of Enterobacteriaceae isolated from serum samples that recorded a significant widal titre value

Age (Years)	No. of Significant widal titre value	No. of Significant widal titre value + Bacterial positive blood culture
0-10	3	0
11-20	3	0
21-30	40	14 (35%)
31-40	3	0
≥41	0	0

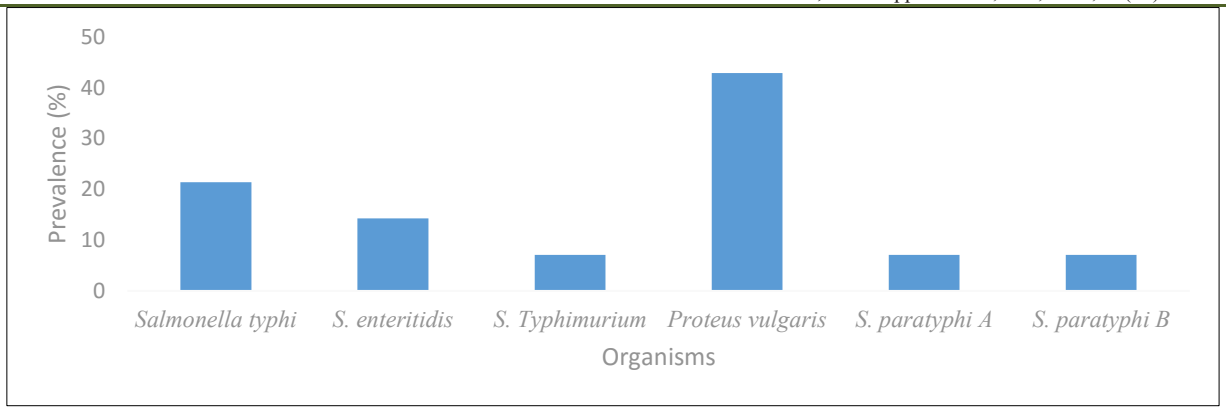

Figure 1: Distribution of bacteria species isolated from blood samples of patients diagnosed of typhoid fever

Table 4: Antibiotic susceptibility profile of bacteria belonging to Enterobacteriaceae family isolated from blood of typhoid patients

Enterobacteriaceae	No. of isolates	Susceptibility of bacterial isolates to the antibiotics									
		OFX	PEF	CPX	AU	GN	ST	CPE	NA	SXT	PN
Proteus vulgaris	6	S	S	S	S	S	S	2(33%)	4(67%)	S	4(67%)
S. paratyhpi A	1	S	S	S	S	S	S	S	S	S	S
S. paratyphi B	1	S	S	S	S	S	S	S	S	S	S
S. typhi	3	S	S	S	S	S	S	S	S	S	S
S. enteritidis	1	S	S	S	S	S	S	S	S	S	S
S. typhimurium	2	S	S	S	S	S	S	S	S	S	S

Keys: OFX-Tarivid (10µg), PEF-Reflacine (10µg), CPX-Ciproflox (10µg), AU-Augmentin (30µg), GN-Gentamycin (10µg), ST-Streptomycin (30µg), CPE-Ceporex (10µg), NA-Nalidixic acid (30µg), SXT-Septrin (30µg), PN-Ampicillin (30µg), S-Sensitive.

DISCUSSION

This study showed that 40.8 % of the patients (n=120) screened for typhoid fever using widal test kit recorded significant titre values. Serology tests revealed that the aetiologic agents were *Salmonella paratyphyi* A, *S. paratyphi* B, and *Salmonella typhimurium*, whereas non-typhoid Salmonella (NTS) found in the specimen was *S. enteritidis*. Further analysis revealed that 28.6 % of the samples that recorded significant titre values (widal test) were infected with another bacterium belonging to the Enterobacteriaceae family which was identified as *Proteus vulgaris*. This bacterium was found in the serum samples of both male and female patients diagnosed with typhoid fever. *Proteus vulgaris* was detected in the blood specimen of more females (n=5) than the male patients (n=1). This result is not in agreement with the report by Priya *et al.,* (2022) It could be attributed to differences in the region where both studies were conducted (Priya *et al.,* 2022). According to Obadire *et al.,*(2022), *Proteus vulgaris* is a pathogen that cause opportunistic infections in human. Immunocompromised persons and patients who have spent a long time in hospitals and care facilities stand a high risk of becoming infected by *Proteus vulgaris* (Obadire *et al.,*2022).

After culturing the serum specimens obtained from two female patients diagnosed with typhoid fever based on widal screening, biochemical tests revealed that the patients were also infected with *Salmonella enteritidis*. This bacterium was not detected in the male patients. *Salmonella enteritidis* is a non-typhoidal Salmonella (NTS) responsible for gastroenteritis. In African countries, *Salmonella enteritidis* is known to cause invasive disease (Worley, 2023). According to Ali *et al.,*(2020), persistent bacteremia in adults due to *S. enteritidis* could cause endocarditis, arteritis or an infected aortic aneurysm.

The highest number of serum samples (40) that recorded a significant titre values involved patients between 21-30 years. Of the 40 patients, serum samples from fourteen (14) patients were observed with microbial growth in blood culture, which represent the highest frequency of occurrence among the age brackets. In a related study that involved isolation of *Proteus vulgaris* from blood and wound specimens, Priya *et al.,*(2022) reported that hospitalized patients in a hospital between 21-35 years had the highest frequency of occurrence (7). Meanwhile, the frequency of occurrence of *Proteus vulgaris* isolates from wound and blood specimens is 11 and 9, respectively. Among the bacteria that belong to Enterobacteriaceae family isolated from the patients, *Proteus vulgaris* had the highest prevalence (42.90 %), while *Salmonella typhimurium, S. paratyphi* A and *S. paratyphi* B had the lowest (7.10 % each) (Figure 1).

Although potentially pathogenic bacteria belonging to Enterobacteriaceae family were isolated

from 49 out of 120 patients, the antimicrobial susceptibility test showed that majority of the isolates were sensitive to the antibiotics tested except *Proteus vulgaris* which was resistant to ceporex, nalidixic, and ampicillin. The highest number of *P. vulgaris* isolates were resistant to nalidixic acid (n=4) and ampicillin (n=4), while the least is ceporex (2). All the bacterial isolates (*Proteus vulgaris, Salmonella typhimurium*, *Salmonella paratyphi* A, *S. paratyphi* B, *S. typhi*, and *S. enteritidis*) were sensitive to tarivid, gentamycin, reflacine, ciproflox, augmentin, streptomycin, and septrin. This report is encouraging because of increasing concern raised by some researchers regarding the spread of antibiotic resistance among bacteria belonging to Enterobacteriaceae family. Widespread antibiotic resistance increases the risk of treatment failure, prolonged hospitalization, and death in extreme cases.

CONCLUSION

Patients clinically diagnosed with typhoid fever by widal screening could also be infected with other bacteria belonging to Enterobacteriaceae family. Therefore, culturing of blood samples of patients who recorded a significant titre value from widal test is required. The bacterial isolates should be identified and subjected to antibiotic susceptibility test. Although the routine laboratory practice to diagnose blood stream infections caused by bacteria belonging to Enterobacteriaceae family will take few days to complete, the result obtained is helpful to provide the patient with an effective antibiotic treatment.

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Data availability: Data for this study will be made available on request

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